Flowering of *Aldrovanda vesiculosa* in Outdoor Culture in the Czech Republic and Isozyme Variability of its European Populations

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Keywords: chemistry: plant — cultivation: *Aldrovanda*.

Introduction

Aldrovanda vesiculosa L. (Droseraceae) is a rootless aquatic carnivorous plant which occurs on every continent in the Old World, but which is rapidly vanishing from Europe (Berta, 1961; Huber, 1961; Degreef, 1986; and Adamec, 1995a). The majority of recently studied sites are in Poland (Adamec, 1995a). *Aldrovanda* grows in shallow, standing, dystrophic waters. Both its extreme rarity and difficult cultivation have caused the species to be little studied. Recently, extensive ecophysiological research on *Aldrovanda* (Adamec, 1997, 1996c) has been accomplished in the Czech Republic. Furthermore, an experimental selection of suitable substitute growth sites has been performed (Adamec, 1996b). Both studies required many cultivated plants. However, cultivation of *Aldrovanda* has only been described on a rudimentary level (e.g., Hanabusa, 1974; Pietropaolo and Pietropaolo, 1986) and no data on water chemistry have been published.

European temperate-zone populations of *Aldrovanda* flower very rarely in natural sites (Berta, 1961; Degreef, 1986; Teryokhin, 1986) whereas subtropical populations flower richly and bear seeds frequently (Pietropaolo and Pietropaolo, 1986; Nakano, 1992). In the Institute of Botany at Wroclaw University, Poland, herbarium specimens with flowering *Aldrovanda* from the following European countries are deposited: south Poland (five sites in Silesia), north and northeast Italy, Romania (Danube delta), Serbia (Yugoslavia), and northeast Turkey. The plants have also flowered in north Russia near Lake Ladoga (Afanas'ev, 1953), in the northwest tip of Ukraine (Teryokhin, 1986), in east Slovakia (Berta, 1961; Studnicka, 1984), south Bavaria, Germany (Huber, 1961), southwest France, and in central Italy (Caspary, 1862, cit. Degreef, 1986). *Aldrovanda* was rarely observed to flower in nearly all European countries of its former distribution. However, fruits and seeds usually fail to develop in flowering plants in Europe (Caspary, 1862, cit. Degreef, 1986; Teryokhin, 1986). The European plants of this species have never flowered in cultivation (Studnicka, 1984).

The origins of Eurasian temperate populations of *A. vesiculosa* and their relationships to tropical African ones are still controversial (Berta, 1961). On the basis of palaeobotanical findings and turion formation, this species is considered to be a Tertiary relict in the European flora (Berta, 1961; Degreef, 1986). The European distribution of *Aldrovanda* was highly discontinuous, occurring in small isolated patches, scattered or grouped together. *Aldrovanda* is spread by migratory water birds and this may explain why its former European distribution overlapped with main migratory routes of water birds (Berta, 1961).

In this paper, characteristics of the flowering plants of European *Aldrovanda vesiculosa* in outdoor culture in Trebon (latitude 49° N, Czech Republic) are presented. Isozyme variability among four European populations is also compared.

Materials and Methods

Aldrovanda was collected in east Poland in June 1993. To maintain the plants in cultivation, conditions existing at the species' most prolific natural sites in Europe were mimicked (Adamec, 1995a). These conditions resembled those described by Hanabusa (1974). Cultivation techniques are fully described in another paper in this issue of CPN (see p85-88).

Aldrovanda plants of three other European populations (northeast Poland; Switzerland, here introduced from Lake Constance in south Germany; and Italy, possibly Lake Sibolla near Lucca in central Italy) were cultivated outdoors in aquaria three to twenty liters in size. The aquaria, covered by glass panes, stood in a plastic container (2.5 m^2) filled with 0.6 m of water to provide cooling. Water in the plastic container was stirred by bubbled air throughout the summer to prevent the aquaria from overheating. In addition, the aquaria were also shaded by plastic foil. The water levels in the aquaria were maintained 1—2 cm lower than that in the plastic container.

Flowering cultivated plants were investigated from July to September 1994. At the beginning of flowering (July 23), all fifty flowering plants (out of a total of 250 plants) were analysed. For each plant, measurements were made of the total length, the number of adult leaf whorls on the main shoot, the number of flowers and flower buds, and the number of shoot branches. The total length of each flower stalk was measured only in fully developed flowers or those in subsequent stages of capsule development.

At the peak of plant flowering (August 15), the number of flowers and fruits including flowers damaged by parasites were estimated in all 138 flowering plants (out of about 500 plants). Four categories of flower and fruit development were defined: A—young flower buds with stalks shorter than 1 cm; B—flower buds with stalks longer than 1 cm and flowers just before opening or fully opened; C—young green capsules with green sepals; D—old brownish capsules with brownish sepals (evidently abortive capsules). On September 28, twenty-one fertile capsules were harvested and the numbers of seeds were estimated.

Polyacrylamide gel electrophoresis (PAGE) has been used according to Kirschner *et al.* (1994); for systems of enzyme detection see Vallejos (1983) and Kirschner *et al.* (1994). Adult plants of *A. vesiculosa* were hand-cleaned of filamentous algae and prey carcasses in traps. The apical shoot segment (1.5 cm long, fresh weight approximately 70 mg) of each plant was ground in an ice-cold extraction buffer. Crude homogenates were centrifuged at 20,000 rpm for ten minutes. The clear supernatant was immediately subjected to gel electrophoresis.

The following enzyme systems were investigated: malate dehydrogenase (three loci), NADH dehydrogenase (five loci), alcohol dehydrogenase (two loci), glutamate dehydrogenase (one locus), phosphogluconate dehydrogenase (two loci), aspartate aminotransferase (two loci), and phosphoglucomutase (one locus).

Results and discussion

Temperature maxima in the cultivation medium close to the surface was usually 26—32°C during July 1994, which induced prolific flowering. Temperatures fell to 14—23°C in September but the plants still flowered and produced new flower buds. On the basis of 1994 and 1995 seasons, the minimum afternoon water temperature needed to induce flowering in *Aldrovanda* from east Poland may be assumed to be 26—28°C for 2—3 weeks (cf. Studnicka, 1984; Degreef, 1986). Presumably, good CO_2 content in the water, prey availability, and irradiance of 40% full daylight or greater are other requirements for flowering.

The first flower buds appeared on July 19, 1994, and opened flowers were observed from July 22 until September 19. The flowering plants were usually 7.8—9.9 cm long and all were branched (Table 1). The branches prevented the shoots with flowers from rotating and stabilized the opened flowers above the water sur-

face. The adult flower stalks were only 1.4—1.6 cm long. The diameters of fully opened flowers were 6—8 mm. At the end of July, the majority of flowers opened while only a minor number did at the end of August and September. Most of the tardily developing flower buds did not ripen. As observed by Berta (1961) in east Slovakia, *Aldrovanda* formed chasmogamous (i.e., opened) flowers only under optimal habitat conditions and cleistogamous (closed), abortive flowers under suboptimal ones. In our cultivation, the flowers opened shortly for only two to three hours, between 3:00 p.m. and 6:00 p.m. The opened flowers floating on the water surface were susceptible to soaking. Caspary (1862, cit. Degreef, 1986), however, reported that the flowers in Poland opened from 7:45 am to 7:00 p.m. How pollination of the flowers occurs is unknown (Pietropaolo and Pietropaolo, 1986). In our culture, self-pollination rarely took place (and probably only in chasmogamous flowers). The low pollination efficiency is probably due to the short time during which the flowers are opened, and their susceptibility to soaking.

At peak flowering (August 15), all 260 flowers at various stages of development were analysed (Table 2). The mean number of flowers per plant was 1.88 with a maximum of five. Eighty-nine percent of the plants bore one to three flowers. Degreef (1986) reported a maximum of three flowers per main shoot. This value reflects not only the number of flower buds per inflorescence, but also the rates of shoot growth and decomposition. On August 15, all stages of flower development were present nearly evenly, with slightly more adult flowers just before or after opening (Table 2). Parasitized flowers occurred most often in the old abortive capsules. The agents were probably diminutive insect larvae.

Of all flowers, only twenty-one capsules bore seeds. The abortive capsules were brownish and bore soft abortive ovules around 0.6 mm long. The capsules with ripe seeds (length 4.0—5.5 mm, width 3.0—4.0 mm) were considerably larger than the abortive ones, turgid, and kept their green colour till the end of September. They contained one to nine hard seeds 1.2—1.4 mm long (mean 4.0 seeds per capsule, median 3.0, quartils 2.0—6.0). The black glossy seeds sink in water. Teryokhin (1986) described 1.5 mm long seeds from northwest Ukraine. Nakano (1992) reported one to seven seeds per capsule in Japanese plants. Although the seeds of European plants may also germinate (Degreef, 1986; Teryokhin, 1986), the germination of seeds is very rare in Europe, but quite common in subtropical or tropical countries (Nakano, 1992). Heat and light promote their germination (Degreef, 1986; Nakano, 1992). In twelve seeds of our harvest, half germinated within one year (Pásek, 1995).

Isozyme analysis of populations from northeast and east Poland, Italy, and Germany revealed no variability within or among populations in fifteen of sixteen loci of the seven enzymatic systems tested. However, one NADH dehydrogenase locus was missing in all samples from a northeast Poland population. Although only 5—20 plants were analyzed from each population, no observed isozyme variability within these populations may indicate their clonal character with lack of generative reproduction. The homogeneity found among distant populations (1,300 km between central Italy and east Poland) indicates that most recent European populations of *Aldrovanda* have a common origin. The present distribution is a mere fraction of the historical one (Adamec, 1995a; cf. Berta, 1961; Huber, 1961). We may speculate that the lack of genetic variability of *Aldrovanda* in Europe is caused by the isolation of these marginal populations with little or no contact with the probably more diverse subtropical and tropical populations.

Acknowledgments

The paper is dedicated to Dr. Donald E. Schnell on the occasion of his twenty-fifth anniversary of editing Carnivorous Plant Newsletter and for his whole life study and popularization of carnivorous plants. Thanks are due to Dr. Ryszard Kaminski from Wroclaw University, Poland, for kindly providing the herbarium data and to Stewart Hanson (Arizona, USA) for the language correction. This

research was granted by the Academy of Sciences of the Czech Republic (project No. 605401).

Table 1. The characteristics of fifty flowering *A. vesiculosa* plants on July 23, 1994. The number of adult leaf whorls in the main shoot is given. The numbers of shoot branches, flowers and flower buds are expressed per plant.

	Median	Quartils	Mean	Range
Main shoot length (cm)	8.7	7.8—9.9	9.0	6.1—15.6
Number of leaf whorls	18.0	17—20	18.8	13—27
Number of shoot branches	1.0	1.0—1.0	1.2	1—2
Number of flowers and buds	1.0	1.0—1.0	1.02	1—2
Adult stalk length (cm)	1.5	1.4—1.6	1.55	1.2—1.9 (n=19)

Table 2. The characteristics of 138 flowering *A. vesiculosa* plants on August 15, 1994. (A) number of plants with the given numbers of flowers and flower buds expressed in % of all 138 plants; (B) categories of flower development in single plants: I, young flower buds with stalks shorter than 1 cm; II, flower buds with stalks longer than 1 cm and flowers just before or after opening; III, young green capsules with green sepals; IV, old brownish capsules with brownish sepals; (C) relative occurrence of damaged flowers in the four categories.

(A)	Number of flowers and buds per plant				
	1	2	3	4	5
Occurrence	50.0%	23.3%	15.9%	10.1%	0.7%
Flowers/plant: 1.5 (median), 1.0—3.0 quartils, 1.88 (mean).					
(B)	Categories of flower development				
	I	II	III	IV	
Occurrence in plants	40.6%	60.1%	34.8%	31.2%	
% of all flowers	21.5%	33.1%	23.1%	22.3%	
(C)	Categories of flower development				
	I	II	III	IV	
Damaged flowers of all flowers in the category	1.8%	8.1%	5.0%	19.0%	

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